



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY  
AND POLLUTION PREVENTION

September 18, 2014

MEMORANDUM

**Subject:** Review of ASRI studies for Copper Sulfate

PC Code: 025601	DP Barcode: D415848, D417513
Decision No. 486968	Registration No.: N/A
Petition No.: N/A	Regulatory Action: Review of two ASRI studies for copper sulfate
Risk Assess Type: None	Case No.: N/A
TXR No.: N/A	CAS No.: 1344-73-6 (basic copper sulfate)
MRID Nos.: 49277401, 49224801	40 CFR: N/A

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**Thru:** Laura Parsons, Senior Interdisciplinary Scientist  
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**To:** Miguel Zavala, Chemical Review Manager  
Risk Management and Implementation Branch III  
Antimicrobials Division (7508P)

INTRODUCTION:

The American Chemistry Council (ACC) and the Copper Sulfate Task Force have submitted two Activated Sludge Respiration Inhibition (ASRI) studies (MRIDs 49277401, 49224801). Neither study included raw data by which the Agency can verify the individual 50 % Inhibition values (IC<sub>50</sub>s) but the studies can still be reviewed and evaluated. For copper that is not nano-scale, the OCSP 850.3300/850.6800 data requirement is satisfied with the combination of these two studies that are supplemental and may be used for risk assessment.

The Data Evaluation Records (DERs) for MRIDs 49224801 and 49277401 are attached.

## Data Evaluation Record 1

### ACTIVATED SLUDGE RESPIRATION INHIBITION OF COPPER

**Report:** Song, J.S., D.K. Cha, E. A. Herbert, A. Son, and J.W. Choe. September 23, 2013. Effect of Copper on Nitrifying and Heterotrophic Populations in Activated Sludge. Unpublished study submitted by the Copper Sulfate Task Force, Valdosta, GA.

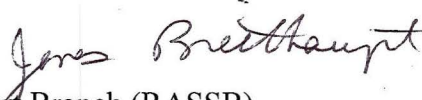
**Document No.:** MRID 49224801

**Guideline:** OCSP 850.3300, 850.6800

**Classification:** The Song et al (2013) study for activated sludge respiration inhibition (ASRI) for copper provides supplemental data and partially satisfies the 850.3300/850.6800 data requirement for copper that is non nanoscale.

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### EXECUTIVE SUMMARY

For all treatments, 50 % inhibition of nitrifiers occurred. For the 1, 3, 6, and 15 mg/l treatments, 50 % inhibition occurred by 3, 2.8, 2, and 0.8 days after treatment, respectively. However, the 50 % inhibition value (IC<sub>50</sub>) for heterotrophs only occurred for the 6 and 15 mg/l treatments at 6 and 1 days after treatment, respectively. The average IC<sub>50</sub> values for "total" copper were 1 and 6 mg/l for nitrifiers and heterotrophs, respectively. The registrant also presented an IC<sub>50</sub> of "free" copper to nitrifiers and heterotrophs, which was  $6.4 \times 10^{-4}$  mg/l for nitrifiers and  $2 \times 10^{-4}$  mg/l for heterotrophs.

### I. MATERIALS AND METHODS

**Guideline followed:** OCSP 850.3300/850.6800.

**GLP Compliance:** The submitter of the study neither sponsored this study nor conducted it and does not know if the study was conducted in accordance with 40 CFR 160 Good Laboratory Practices.

## A. Materials:

1. **Test Material:** Copper sulfate (reagent grade or better)

## 2. Methods:

Samples of copper sulfate at 1, 3, 6, and 15 mg/l copper ion were added to bench-scale reactors containing activated sludge, primary effluent, and synthetic wastewater. Samples were taken at 1, 2, 3, 5, and 7 days after treatment. Each bench-scale unit consisted of an aeration basin and a secondary clarifier with continuous flow of primary effluent of 3.4 L/day at 28 °C for 15 days. Ammonia, total organic carbon (TOC), and free (ionic) copper were measured in the study to determine the effect of copper on nitrifiers and heterotrophs. The Mean Cell Residence Time (MCRT) was 15 days, which means that a microorganism is expected to stay in an activated sludge system for an average of 15 days. According to the State of Mississippi Department of Environmental Quality, the typical values of MCRT are usually in the range of 5 to 15 days.<sup>1</sup>

## II. RESULTS AND DISCUSSION

For all treatments, 50 % inhibition of nitrifiers occurred. For the 1, 3, 6, and 15 mg/l treatments, 50 % inhibition occurred by 3, 2.8, 2, and 0.8 days after treatment, respectively. However, 50 % inhibition value (IC<sub>50</sub>) for heterotrophs only occurred for the 6 and 15 mg/l treatments at 6 and 1 days after treatment, respectively. The IC<sub>50</sub> values for “total” copper were 1 and 6 mg/l for nitrifiers and heterotrophs, respectively. The registrant also presented an IC<sub>50</sub> of “free” copper to nitrifiers and heterotrophs, which was  $6.4 \times 10^{-4}$  mg/l for nitrifiers and  $2 \times 10^{-4}$  mg/l for heterotrophs.

## III. REVIEWER COMMENTS

The study did not include negative controls, a reference substance, or raw data for verification of IC<sub>50</sub> values. However, the lack of a negative control does not invalidate the study because inhibition observed in increasing treatments was consistent with higher treatment rates. A reference substance is normally used to determine the accuracy of the results of the compound of interest, but this does not invalidate the study because the results of MRID 49277401 are also consistent with these results. While raw data were not presented, visual examination of the graphs in the study provide the inhibition results presented in the study.

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<sup>1</sup>  
[http://www.deq.state.ms.us/MDEQ.nsf/8f3cca80758d46df86256c080014bab8/557702f42378ab3b862573220073b407/\\$FILE/QP7498-DEQ-Chapter%205%20corrections\\_pg%2054.pdf](http://www.deq.state.ms.us/MDEQ.nsf/8f3cca80758d46df86256c080014bab8/557702f42378ab3b862573220073b407/$FILE/QP7498-DEQ-Chapter%205%20corrections_pg%2054.pdf)

#### **IV. REFERENCES**

State of Mississippi, Department of Environmental Quality.

[http://www.deq.state.ms.us/MDEQ.nsf/8f3cca80758d46df86256c080014bab8/557702f42378ab3b862573220073b407/\\$FILE/QP7498-DEQ-Chapter%205%20corrections\\_pg%2054.pdf](http://www.deq.state.ms.us/MDEQ.nsf/8f3cca80758d46df86256c080014bab8/557702f42378ab3b862573220073b407/$FILE/QP7498-DEQ-Chapter%205%20corrections_pg%2054.pdf)

U.S. EPA. April, 1996. Modified Active Sludge, Respiration Inhibition Test for Sparingly Chemicals, public draft. OCSPP 850.6800. EPA 712-C-96-168.

U.S. EPA. January, 2012. Modified Activated Sludge, Respiration Inhibition Test. OCSPP 850.3300. EPA 712-C-014.



## Data Evaluation Record 2

### ACTIVATED SLUDGE RESPIRATION INHIBITION OF COPPER

**Report:** Cha, D.K., H.E. Allen, and J.S. Song. 2004. Effect of Copper and Nitrifying and Heterotrophic Populations in Activated Sludge. Unpublished study performed by the Department of Civil and Environmental Engineering, University of Delaware, and submitted by the ACC Biocides Panel Copper Task Force. Report No. CTF-2013.02

**Document No.:** MRID 49277401

**Guideline:** OCSPP 850.3300, 850.6800

**Classification:** The Cha et al (2013) study for activated sludge respiration inhibition (ASRI) for copper provides supplemental data and partially satisfies the 850.3300/850.6800 data requirement for copper that is not nanoscale.

**PC Code:** 025601

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Antimicrobial Division (7510P)

### EXECUTIVE SUMMARY

For both nitrifiers and heterotrophs, inhibition of 50 % occurred at higher total copper concentrations. For nitrifiers, the exposure times required for 50 % inhibition were 0.8-0.9 day for 30, 40, and 50 mg/l and 2-3.8 days for the 15 mg/l treatment (15-day MCRT, Figure 3.4, p. 32). As expected, the  $IC_{50}$  decreased as the MCRT increased, declining from approximately 13.4 mg/l at 10 days to 9.9 mg/l by 30 days of MCRT (Figure 3.17, p. 41).

For heterotrophs, the exposure time required for 50 % inhibition increased with decreasing concentration of copper. The exposure times required for 50 % inhibition were 0.5-0.6 day for 40, and 50 mg/l and 2.3 days for the 15 and 30 mg/l treatments (15-day MCRT, Figure 3.5, p. 33). As expected, the  $IC_{50}$  decreased as the MCRT increased, declining from approximately 14.6 mg/l at 10 days to 5.5 mg/l by 30 days of MCRT (Figure 3.17, p. 41).

### I. MATERIALS AND METHODS

**Guideline followed:** OCSPP 850.3300/850.6800.

**GLP Compliance:** The submitter of the study neither sponsored this study nor conducted it and does not know if the study was conducted in accordance with 40 CFR 160 Good Laboratory Practices.

## A. Materials:

1. **Test Material:** Copper sulfate (reagent grade or better)

## 2. Methods:

Bench-scale reactors were constructed according to the OECD 303 test guideline, and these reactors consisted of an aeration basin and a secondary clarifier made of transparent acrylic polymer. Samples of copper sulfate at 0, 3, 6, 15, 30, 40, and 50 mg/l copper ion were added to these reactors containing activated sludge, primary effluent, and synthetic wastewater to achieve a dissolved organic carbon (DOC) of 50 mg/l. Samples were taken at 0, 1, 4, and 6 hours and 1, 2, 3, 5, and 7 days after treatment with mean cell residence times (MCRT) of 10, 12, 15, 20 and 30 days. Each bench-scale unit consisted of an aeration basin and a secondary clarifier with a volume of 3.4 L/day and an inflow of 6.8 L/day at 25 °C for up to 30 days. Ammonia, total organic carbon (TOC), and free (ionic) copper were measured in the study to determine the effect of copper on nitrifiers and heterotrophs.

## II. RESULTS AND DISCUSSION

### Nitrifiers

For nitrification, the only treatments that caused at least 50 % inhibition were 6, 15, 30, 40, and 50 mg/l copper. The exposure time required for 50 % inhibition increased with decreasing concentration of copper. The exposure times required for 50 % inhibition were 0.8-0.9 day for 30, 40, and 50 mg/l and 2-3.8 days for the 15 mg/l copper treatment (15-day MCRT, Figure 3.4, p. 32). As expected, the  $IC_{50}$  decreased as the MCRT increased, declining from approximately 13.5 mg/l at 10 days to 11 mg/l by 30 days of MCRT (Figure 3.17, p. 41).

### Heterotrophs

For heterotrophs, the only treatments that caused at least 50 % inhibition were 15, 30, 40, and 50 mg/l copper. The exposure time required for 50 % inhibition increased with decreasing concentration of copper. The exposure times required for 50 % inhibition were 0.5-0.6 day for 40, and 50 mg/l and 2.3 days for the 15 and 30 mg/l copper treatments (15-day MCRT, Figure 3.5, p. 33). As expected, the  $IC_{50}$  decreased as the MCRT increased, declining from approximately 12 mg/l at 10 days to 5-6 mg/l by 30 days of MCRT (Figure 3.17, p. 41).

Table 1 below contains the  $IC_{50}$  and NOAEC values for total and free copper for both nitrifiers and heterotrophs. Table 2 contains the  $IC_{50}$  and NOAEC values for soluble copper for nitrifiers and heterotrophs at different MCRTs.

**Table 1. IC<sub>50</sub> and NOAEC Values of Total and Free Copper for Nitrifiers and Heterotrophs at different Mean Cell Residence Times (MCRTs).**

MCRT (day)	Nitrifiers				Heterotrophs			
	IC <sub>50</sub> of total copper (mg/L)	IC <sub>50</sub> of free copper (moles/L)	IC <sub>50</sub> of free copper (mg/L)	NOAEC of total copper (mg/L)	IC <sub>50</sub> of total copper (mg/L)	IC <sub>50</sub> of free copper (moles/L)	IC <sub>50</sub> of free copper (mg/L)	NOAEC of total copper (mg/L)
10	13.4	$8.8 \times 10^{-9}$	$5.6 \times 10^{-4}$	4.3	14.6	$5.7 \times 10^{-9}$	$3.6 \times 10^{-4}$	5.4
12	13.8	$7.1 \times 10^{-9}$	$4.5 \times 10^{-4}$	4.1	8.5	$7.4 \times 10^{-9}$	$4.7 \times 10^{-4}$	2.9
15	10.3	$9.9 \times 10^{-9}$	$6.3 \times 10^{-4}$	3.5	9.3	$5.3 \times 10^{-9}$	$3.4 \times 10^{-4}$	3.6
20	13.7	$5.5 \times 10^{-9}$	$3.5 \times 10^{-4}$	4.4	7.3	$1.0 \times 10^{-9}$	$6.4 \times 10^{-5}$	2.5
30	9.9	$3.6 \times 10^{-9}$	$2.3 \times 10^{-4}$	3.5	5.5	$7.8 \times 10^{-10}$	$5.0 \times 10^{-5}$	2.0

**Table 2. IC<sub>50</sub> and NOAEC Values of Soluble Copper for Nitrifiers and Heterotrophs at different Mean Cell Residence Times (MCRTs).**

MCRT (day)	Nitrifiers		Heterotrophs	
	IC <sub>50</sub> of soluble copper (mg/L)	NOAEC of soluble copper (mg/L)	IC <sub>50</sub> of soluble copper (mg/L)	NOAEC of soluble copper (mg/L)
10	0.74	0.26	1.15	0.45
12	0.87	0.27	0.61	0.26
20	0.91	0.29	0.51	0.23

### III. REVIEWER COMMENTS

The study did not include a reference substance or raw data for verification of IC<sub>50</sub> values. A reference substance is normally used to determine the accuracy of the results of the compound of interest, but this does not invalidate the study because the results of MRID 49224801 are also consistent with these results. While raw data were not presented, visual examination of the graphs in the study provide the inhibition results presented in the study.

### IV. REFERENCES

State of Mississippi, Department of Environmental Quality.

[http://www.deq.state.ms.us/MDEQ.nsf/8f3cca80758d46df86256c080014bab8/557702f42378ab3b862573220073b407/\\$FILE/QP7498-DEQ-Chapter%205%20corrections\\_pg%2054.pdf](http://www.deq.state.ms.us/MDEQ.nsf/8f3cca80758d46df86256c080014bab8/557702f42378ab3b862573220073b407/$FILE/QP7498-DEQ-Chapter%205%20corrections_pg%2054.pdf)

U.S. EPA. April, 1996. Modified Active Sludge, Respiration Inhibition Test for Sparingly Chemicals, public draft. OCSPP 850.6800. EPA 712-C-96-168.

U.S. EPA. January, 2012. Modified Activated Sludge, Respiration Inhibition Test. OCSPP 850.3300. EPA 712-C-014.